

CLAIMS

1. An HIV vaccine composition characterized in that it comprises at least one stabilized Tat antigen resistant to proteolytic degradation, said stabilized antigen being selected from the group consisting of:
  - a) a complex between an HIV Tat protein or a Tat fragment of at least 11 amino acids, and a non-metal ligand of Tat,
  - b) an HIV Tat protein or a Tat fragment of at least 11 amino acids, modified by substitution with a hydrophobic amino acid and/or the modification, with a hydrophobic group, of at least one amino acid of the Tat sequence, with the exclusion of the substitutions R52L, R55L, R57L, R78A, G79A, E80A and K89L, and
  - c) a complex between the Tat protein or the Tat fragment modified by substitution with a hydrophobic amino acid and/or the modification, with a hydrophobic group, of at least one amino acid of the Tat sequence defined in b), and a non-metal ligand of Tat.
2. The vaccine composition as claimed in claim 1, characterized in that said non-metal ligand in a) or in c) is protein, lipid, carbohydrate, nucleotide or mixed in nature.
3. The vaccine composition as claimed in claim 2, characterized in that said non-metal ligand in a) or in c) is a polysulfated sugar chosen from: dextran sulfate, pentosan polysulfate and polysulfated glycosaminoglycans such as heparin or heparan sulfate.
4. The vaccine composition as claimed in claim 3, characterized in that said heparin is a heparin

having a molecular weight of 15 000 Da or a heparin fragment having a molecular weight of 6000 Da.

- 5    5.    The vaccine composition as claimed in claim 2, characterized in that said non-metal ligand in a) or in c) is the HIV Vpr protein.
- 10    6.    The vaccine composition as claimed in claim 1, characterized in that said Tat protein or said Tat fragment in b) is modified by substitution with a hydrophobic amino acid and/or the modification, with a hydrophobic group, of 1 to 7 cysteines located at positions 22, 25, 27, 30, 31, 34 and/or  
15    37.
- 20    7.    The vaccine composition as claimed in claim 6, characterized in that at least the four cysteines at positions 25, 27, 30 and 31 are substituted with a hydrophobic amino acid and/or modified with a hydrophobic group.
- 25    8.    The vaccine composition as claimed in claim 6 or claim 7, characterized in that said hydrophobic amino acid is selected from the group consisting of: a leucine, a tryptophan and a phenylalanine and/or said hydrophobic group is S-tert-butyl.
- 30    9.    The vaccine composition as claimed in any one of claims 1 to 8, characterized in that said stabilized Tat antigen derives from an inactivated Tat protein or from an inactivated Tat fragment.
- 35    10.   The vaccine composition as claimed in claim 9, characterized in that said inactivated Tat protein or said inactivated Tat fragment comprises the substitution of each of the cysteines at positions 22, 34 and 37 to serines or else the substitution

of each of the arginines at positions 52 and 53 to glutamines.

- 5 11. The vaccine composition as claimed in any one of claims 1 to 10, characterized in that said Tat protein or the fragment of said protein is chosen from: the Tat protein of 101 amino acids, the Tat protein of 86 amino acids, the fragment 1 to 57 of Tat and the fragments of at least 11 amino acids  
10 included in the proteins or fragment above.
12. The vaccine composition as claimed in any one of claims 1 to 11, characterized in that said  
15 stabilized Tat antigen derives from the Tat protein of SEQ ID No. 1 or from a fragment of at least 11 amino acids of this sequence.
13. The vaccine composition as claimed in any one of claims 1 to 12, characterized in that said Tat  
20 protein or said Tat fragment in a) is also complexed with a metal ion chosen from polyvalent cations, preferably divalent cations, such as  $\text{Zn}^{2+}$  or  $\text{Cd}^{2+}$ .
- 25 14. The vaccine composition as claimed in any one of claims 1 to 12, characterized in that said modified Tat protein or said modified Tat fragment in b) or in c) is also complexed with a metal ion  
30 chosen from polyvalent cations, preferably divalent cations, such as  $\text{Zn}^{2+}$  or  $\text{Cd}^{2+}$ .
15. The vaccine composition as claimed in any one of claims 1 to 14, characterized in that said Tat  
35 protein or said Tat fragment is a monomer.
16. The vaccine composition as claimed in any one of claims 1 to 14, characterized in that said Tat

protein or said Tat fragment is an oligomer, preferably a dimer.

- 5 17. The vaccine composition as claimed in claim 16, characterized in that said oligomer, preferably dimer, is formed from the covalent association of said Tat protein and/or of the fragment of said protein by means of an intermolecular disulfide bond involving one of the cysteines at position 10 22, 25, 27, 30, 31, 34 and 37.
- 15 18. The vaccine composition as claimed in claim 17, characterized in that said disulfide bond involves one of the cysteines at position 22, 34 or 37.
- 20 19. The vaccine composition as claimed in claim 18, characterized in that the Tat dimer is formed from the association, by means of a disulfide bridge between the cysteines at position 34, of two Tat proteins or of two Tat fragments of at least 11 amino acids comprising a serine at positions 22 and 37 and a leucine at positions 25, 27, 30 and 31.
- 25 20. The vaccine composition as claimed in claim 16, characterized in that said oligomer, preferably dimer, is formed from the noncovalent association of said Tat protein and/or of the fragment of said protein by means of metal ions, preferably of 30 polyvalent cations, in particular divalent cations such as  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$ .
- 35 21. The vaccine composition as claimed in any one of claims 1 to 20, characterized in that said Tat protein and/or the fragment of said protein, which are optionally modified, are in the form of a polynucleotide or of a recombinant vector encoding said protein and/or said fragment.

22. The vaccine composition as claimed in any one of claims 1 to 20, characterized in that it comprises a pharmaceutically acceptable vehicle and/or an adjuvant and/or a carrier substance.
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23. The vaccine composition as claimed in claim 22, characterized in that it consists of a stabilized antigen as claimed in any one of claims 1 to 20 and a pharmaceutically acceptable vehicle and/or a carrier substance.
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24. The vaccine composition as claimed in claim 22, characterized in that said adjuvant is alumina hydroxide.
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25. A stabilized Tat antigen as claimed in any one of claims 1 to 21, as a vaccine for the prevention and/or treatment of an HIV infection in humans.
- 20
26. The use of a stabilized Tat antigen as claimed in any one of claims 1 to 21, for the preparation of a vaccine for use in the prevention and/or treatment of an HIV infection in humans.
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27. A peptide complex, characterized in that it consists of:
- an HIV Tat protein or a Tat fragment of at least 11 amino acids, modified by substitution with a hydrophobic amino acid and/or the modification, with a hydrophobic group, of at least one amino acid of the Tat sequence, as defined in any one of claims 1, 6 to 12 and 15 to 19, associated with
  - a metal ligand of Tat, as defined in claim 14, and/or a non-metal ligand of Tat as defined in any one of claims 2 to 5.
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28. A protein or peptide fragment, characterized in that it is chosen from an HIV Tat protein or a Tat fragment of at least 11 amino acids, modified by substitution with a hydrophobic amino acid and/or the modification, with a hydrophobic group, of at least one amino acid of the Tat sequence, as defined in any one of claims 1, 6 to 12 and 15 to 19, with the exclusion of the substitutions R52L, R55L, R57L, R78A, G79A, E80A and K89L.
29. A method of preparing a stabilized Tat antigen, characterized in that it comprises at least:
- the preparation, by any appropriate means, of an HIV Tat protein or of a Tat fragment as defined in any one of claims 1, 9 to 18 and 21, and simultaneously or sequentially,
  - the formation of a complex with a non-metal ligand of Tat as defined in any one of claims 2 to 5 and/or the substitution, with a hydrophobic amino acid, and/or the modification, with a hydrophobic group, of at least one of the amino acid residues of the Tat sequence, as defined in any one of claims 1, 6, 7 and 8.
30. A polynucleotide or a mixture of polynucleotides selected from the group consisting of:
- a) a polynucleotide or a mixture of polynucleotides comprising the sequence encoding an HIV Tat protein or a Tat fragment of at least 11 amino acids as defined in any one of claims 1, 6 to 13 and 15 to 19, and the sequence encoding a peptide ligand of Tat, and
  - b) a polynucleotide comprising the sequence encoding an HIV Tat protein or a Tat fragment of 11 amino acids, modified by substitution, with a hydrophobic amino acid, of at least one amino acid of the Tat sequence, as defined in any one of claims 1, 6 to 12 and 15 to 19, with the exclusion

of the substitutions R52L, R55L, R57L, R78A, G79A, E80A and K89L.

- 5 31. A recombinant vector comprising the polynucleotide as defined in a) or in b) of claim 30.
32. A mixture of recombinant vectors each comprising polynucleotides of the mixture as defined in a) of claim 30.
- 10 33. A eukaryotic cell modified with a polynucleotide, a recombinant vector or a mixture as defined in any one of claims 30 to 32.
- 15 34. The use of a metal ion as defined in claim 13 or 14, for stabilizing the HIV Tat protein or a fragment of at least 11 amino acids of said protein.